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Effects of Aqueous *Colocasia esculenta* Extracts on Selected Biochemical Parameters in Phenyl Hydrazine Induced Male Anemic Albino Rats

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Authors' contributions

This research work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: In this study, the effect of *Colocasia esculenta* a hematinic plant on biochemical parameters levels (Direct, Total and unconjugated bilirubin, creatinine, total protein, serum albumin and urea) was assessed to determine if the plant extract can reverse the abnormality in the values of these parameter.

Methodology: The experimental animals were divided into four groups as follows; group 1(non-anemic control), group 2 (anemic untreated), group 3 (anemic treated with low dose of plant extract 100 mg/ml), group 4 (anemic treated with high dose of plant extract 500 mg/ml). Anemia was induced in group 1, group 2 and group 3 with 60 mg/kg of phenyl hydrazine for 2 days. After induction of anemia group 2 and group 3 was treated with 100 mg/kg and 500 mg/kg of plant extract for 7 days. After 7-day blood sample was collected through heart puncture and centrifuged for serum. Then, Bilirubin, creatinine, total protein, serum albumin and urea test were carried out.

Results: The anemic untreated group had the highest bilirubin, creatinine and urea value of 1.3 mg/dl, 3.97 mg/dl and 71.78 g/l respectively compared to the non-anemic control (bilirubin-0.4

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mg/dl, creatinine-3.13 mg/dl and urea 60.35 mg/dl), anemic treated with low dose (bilirubin-0.37 mg/dl,creatinine-1.40 mg/dl and urea-41.82), and anemic treated with high dose (bilirubin-0.25 mg/dl, creatinine-0.86 mg/dl, and urea-48.66 mg/dl) with significant increase in phenyl hydrazine value at $p<0.05$ .The anemic nontreated group experienced a reduced value of total protein and albumin of 49.78 g/l and 24.46 g/l respectively compared to the non-anemic control (total protein-66.2 g/l and albumin-37.67 g/l) ,anemic treated with low dose (total protein-67.5 g/l and albumin-19.2 g/l), and anemic treated with high dose (total protein-21.9 g/l and albumin-81.6 g/l).

Conclusion: The obtained results from this study revealed the anti-anemia potentials of aqueous extracts of *Colocasia esculenta.*

Keywords: Colocasia esculenta; phenyl hydrazine; anemia; bilirubin; creatinine; urea; total protein serum albumin.

1. INTRODUCTION

A marked decrease in the oxygen binding ability of each hemoglobin molecule due to inadequate population and deformity of its structure is what is referred to as anemia. Also reduction in hemoglobin and hematocrit level with respect to age, sex and location of an individual [1]. Anemia can be of different types which includes the following: pernicious anemia, which is characteristic to deficiency of vitamin B_{12} (cobalamin) and other intrinsic factors which are needed for erythropoiesis [2]. Sickle cell anemia which is characterized by polymerization of abnormal sickled erythrocytes when hemoglobin is deoxygenated (This polymerization within the red cells causes it to lose form become rigid and obstruct blood flow); Hemolytic anemia, which is the marked imbalance in the erythropoiesis and destruction of red blood cells [3].

Hemolytic anemia is characterized by reticulosis, and the presence of increased un conjugated bilirubin and lactate dehydrogenase , decreased haptoglobin [4]. The liver being a highly vascularized organ is impacted by hemolysis also, it being the second organ of RBC fragment clearance and hemoglobin and heme detoxification makes it prone to stress or damage [5]. Also Merle et al. [6] introduced the linkage between phenyl hydrazine triggered intravascular hemolysis and kidney injury or impairment and reported an increased urea , creatinine and total protein level Criswell et al. [7] reported increased erythropoietin level in plasma, urine, kidney and liver of phenyl hydrazine treated rat showing increased erythropoietic activities.

The application of plant extract to the treatment of anemia has been a topic of scientific interest. Plant extracts like *Justicia canea, Soghurm bicolor* and *Colocasia esculenta* has been of good use. *Colocasia esculenta*, the plant of interest in this study is also known as cocoyam (or taro) [1]. Aside its antianemia properties, Akter et al. [8] reported its anti-hyperglycemic effect in glucose –induced hyperglycemicrat. Its phytochemical constituent and its nutritional value have been linked to its various application. It was discovered to have nutritional value of moisture (6.54%), ash (2.44%), fiber (3.01%), protein (7.79%), fat (0.65%), carbohydrate (86.11%) [9]. Its phytochemical compositions are high level of alkaloids (antifever and head ache reliever) and saponins (treat fungal and yeast infection), flavonoids (radical scavenger and anti-inflammatory), terpenes (anti-hyperglycemic, anti-cancer, anti-allergic, and immune modulatory function) and phenols (antioxidant) Krishnapriya et al. [10]. It was also reported to have minerals such as calcium, phosphorus, iron, vitamin c, thiamine, riboflavin and niacin [1].

These plant extract asides their benefit has effect on some biochemical parameters in the blood. Parameters like creatinine, urea, albumin and bilirubin are going to be studied. Creatinine are nitrogenous product of catabolism of muscle creatinine with small molecular weight of about 133 Dalton [11]. Creatinine are also endogenous substances used as a marker for the determination of kidney function by showing the glomerular filtration rate (GFR). GFR which is inversely related to creatinine level helps to know how well the glomerular filtrate filter out this endogenous substance [12]. Urea are primary metabolites derived from dietary protein and tissue protein turn over [11].

Urea level along with creatinine work hand in hand to determine the glomerular filtration rate of an individual that is to large extent determine the functioning of the kidney [12]. Bilirubin mostly recognized as the end product of metabolism and in high concentration in the serum are piled up in the brain leading to medical condition known as kernicterus [13]. When heme catabolism occurs in the reticuloendothelial cell,

bilirubin are created in their unconjugated form then they are passed to the plasma where they are bound tightly to albumin for uptake by the liver (they either bind to one or two molecules of glucuronide for increased polarity and easy secretion into the bile) [14].

Serum albumin is the most abundant protein in human plasma it's a macromolecule that is monomeric with multiple domain, and it represent the main determinant of plasma oncotic pressure (osmotic pressure that moves water molecule into the circulatory system induced by plasma proteins) and regulate the distribution of fluid in different body parts [15].

Total serum protein assay measures the amounts of two major groups of proteins in the blood: albumin and globulin [16]. Albumin is made mainly in the liver. It helps keep the blood from leaking out of blood vessels. Albumin also helps carry some medicines and other substances through the blood and is important for tissue growth and healing [17]. Globulin is made up of different proteins called alpha, beta, and gamma types. Some globulins are made by the liver, while others are made by the immune system [18]. Certain globulins bind with hemoglobin. Other globulins transport metals, such as iron, in the blood and help fight infection. Serum globulin can be separated into several subgroups by serum protein electrophoresis [19].

Phenyl hydrazine is a yellowish oily liquid compound with a molecular formula of $C_6H_8N_2$ with a molar mass of 108 g/m used to induce anemia in animals during experiments and later used for treatment of polycythemia (increase in entire amount of body erythrocyte) [20]. They induce hemolysis using mechanisms ranging from sulfhydryl group interaction, inhibiting various enzymes, immune mechanism, and finally fragmentation of erythrocyte as they pass through platelet fibrin mesh Berger et al. and Ogbe et al. [20,21] reported its repressing effect on bilirubin level which was later treated with anti-anemic plant extract.

2. MATERIALS AND METHODS

2.1 Plant Identification

Leaves of *Colocasia esculenta* was obtained from a local market in Ojo town Lagos, Nigeria. The plant was identified by the Department of Botany at Lagos state university.

2.2 Reagents and Equipment

Phosphate buffer saline, phenyl hydrazine, serum albumin kit, creatinine kit, urea kit bilirubin kit, water bath, high speed bench centrifuge spectrophotometer, syringe and needle, cannular, anticoagulant blood sample bottle, test tube, beaker, spatula, cottonwool, test tuberack, dissectingset, micropipette, spectrophotometer, glass cuvette, and centrifuge.

2.3 Preparation of Plant Extract

Healthy selected leaves were deveined (by carefully tearing off the vein) and properly rinsed. The wet weight of the leaves was determined and was then spread out to air dry in the laboratory room. Once completely dry, the leaves dry weight was determined and then taken to the miller for grinding after which was sieved to remove chaffs. 200 g of the relevant portion of the grinded leaf was then extracted by decoction in 3 liters of distilled water and filtered using muslin cloth. Impurities from the extract were further removed by centrifugation. The pure plant extract was then concentrated in a water bath at 60°C.

2.4 Lethal Dose Toxicity

Toxic dose of the plant extract was determined by examining their LD_{50}Values. It was carried out by giving them extremely high dose of 800 mg/kg, 2000 mg/kg, 5000 mg/kg *Colocasia esculenta*.

2.5 Pre-Induction of Anemia

A total of two rats were randomly selected and was administere dphenyl hydrazine (PHZ). Before the exposure of the rats to phenyl hydrazine, two rats were sacrificed to determine the basal hemoglobin level of the rat and 13 g/dl was used as the reference value for hemoglobin. Induced 60 mg/kg of phenyl hydrazine was administered to the rat for 2 days. The two rats were sacrificed on the second day and their hemoglobin level were determined to know if PHZ has induced anemia. after test their hemoglobin and PCV level was 8.7 g/dl, 17% and 8 g/dl ,15% respectively.

2.6 Animal Grouping

40 male albino rats weighing between 180 g to 240 g were procured for experiment. The experimental animals were acclimatized in the department of biochemistry animal house for the

period of two weeks under natural hours of day light and night condition, all the animals were fed with standard rat diet and water.

Group 1: Non anemic control group.
Group 2: Anemic untreated.
Group 3: Anemic treated with 100 mg/kg of *Colocasia esculenta* (low dose).
Group 4: Anemic treated with 500 mg/kg of *Colocasia esculenta* (high dose).

2.7 Induction of Anemia and Treatment of Anemia

After acclimatization, rats were randomly divided into four (4) groups with the exception of group one (1) which serves as the control group, rats in the remaining three (3) were administered with 60 mg/kg of phenyl hydrazine .

100 mg/kg and 500 mg/kg of *Colocasia esculenta* extract was administered to the low dose group and high dose rat respectively to restore normality to the effect of phenyl hydrazine.

2.8 Sacrifice of Experimental Animals

Exactly after 7 days of treatment administration to selected group of the experimental animal the animals were anaesthetized with ethyl ether to enable easy dissection of the animal and blood sample was carefully collected through heart puncture from each group into a heparinized tube to avoid coagulation.

2.9 Biochemical Assays

2.9.1 Direct bilirubin test

Following standard commercial test kit guideline obtained from RANDOX laboratories 200 µl sera samples of the sacrificed rats were mixed with 200µl of sulphanilic acid and 50 µl of nitrite, 2000 µl of 0.9% NaCl and incubated for 10 minutes at 20-25°C. likewise, a sample blank was prepared with the same reagents (minus nitrite) and the samples' absorbance were read at 546nm with a spectrophotometer.

2.9.2 Total bilirubin test

Following standard commercial test kit guideline obtained from RANDOX laboratories. 200 µl sera samples of the sacrificed rats were mixed with 200µl of sulphanilic acid and 50 µl of nitrite, 1000 µl of caffeine and 200 µl tartrate and incubated for 30 minutes at 25°C. likewise a sample blank was prepared with the same reagents (minus nitrite) and the samples' absorbance were read at 578nm.

2.9.3 Unconjugated bilirubin test

The results obtained from the total and direct bilirubin test was used to derive the value of unconjugated bilirubin. The formula used was;

Unconjugated Bilirubin(mg/dl) =Total Bilirubin – Direct Bilirubin (mg/dl).

2.9.4 Total protein test

Following standard commercial test kit guideline obtained from RANDOX laboratories. Reagent blank, standard and sera samples were prepared with 20 µl of distilled water, standard CAL and sera samples mixed in with 1 ml of biuret reagent respectively in different tubes and incubated for 30 minutes at 20-25°C and the absorbance of the sample (A_{sample}) and of the standard ($A_{standrad}$) is measured against the reagent blank with a spectrophotometer at 546nm.

2.9.5 Albumin test

Following standard commercial test kit guideline obtained from RANDOX laboratories. Reagent blank, standard and sera samples were prepared with 3µl of distilled water, standard CAL and sera samples mixed in with 3 ml of BCG concentrate respectively in different tubes and incubated for 20 mins at 37°C and the absorbance of the sample (A_{sample}) and of the standard ($A_{standrad}$) is measured against the reagent blank with a spectrophotometer at 630nm.

2.9.6 Serum urea test

Following standard commercial test kit guideline obtained from RANDOX laboratories. Reagent blank, standard and sera samples were prepared with 5 µl of distilled water, standard CAL and sera samples mixed in with 1 ml of sodium nitroprusside and urease solution respectively in different tubes and incubated at 37^oC for 10 minutes and phenol and sodium hypochlorite were mixed in immediately and incubated for 15 minutes at 37^oC.

2.9.7 Creatinine test

Following standard commercial test kit guideline obtained from RANDOX laboratories. Reagent blank, standard and sera sample were prepared

with 100 µl of distilled water , standard CAL and sera sample mixed in with 1 ml of sodium hydroxide and piuric acid respectively in different test tubes and read at two different time frame to get two absorbance values (the difference between the absorbance was gotten). The readings were done at 592 nm.

2.10 Statistical Analysis

Statistical Analysis was performed using GraphPad Prism 7 statistical package (GraphPad Software, USA) and Microsoft Excel. The data were analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni tests. All the results were expressed as Mean+SE for triplicate determinations. Significance was accepted at $P \leq 0.05$.

3. RESULTS

3.1 Direct Bilirubin Levels

Administration of phenyl hydrazine to the albino rat significantly increased its direct bilirubin levels from 0.35 mg/dl to 0.68 mg/dl, however treatment with 100 mg/kg and 500 mg/kg of *Colocasia esculenta* greatly reduced it to 0.25 mg/dl and 0.2 mg/dl respectively (Fig. 1).

3.2 Total Bilirubin Levels

Administration of phenyl hydrazine to the albino rat significantly increased its total bilirubin levels from 0.44 mg/dl to 1.32 mg/dl, and on treatment with 100 mg/kg and 500 mg/kg of *Colocasia esculenta*, it was significantly reduced to 0.37 mg/dl and 0.25 mg/dl respectively (Fig. 2).

3.3 Unconjugated Bilirubin Levels

Administration of phenyl hydrazine to the albino rat significantly reduced its unconjugated bilirubin levels from 0.09 mg/dl to 0.64 mg/dl and treatment with 100 mg/kg and 500 mg/kg of *Colocasia esculenta* significantly reduced it to 0.12 mg/dl and 0.04 mg/dl respectively (Fig. 3).

3.4 Creatinine Levels

Administration of phenyl hydrazine to the albino rat did not significantly increase its creatinine level from 3.13 mg/dl to 3.97 mg/dlbut treatment with 100 mg/kg and 500 mg/kg of *Colocasia esculenta* significantly reduced it to 1.40 mg/dl and 0.86 mg/dl respectively (Fig. 4).

3.5 Total Protein Levels

Administration of phenyl hydrazine to the albino rat significantly reduced its total protein level from 66.2 g/l to 49.78 g/land on treatment with 100 mg/kg and 500 mg/kg of *Colocasia esculenta* significantly increased it to 67.47 g/l and 81.62 g/l respectively (Fig. 5).

3.6 Serum Urea Levels

Administration of phenyl hydrazine increased the serum urea level of the albino rat from 60.35 g/l to 71.78 g/l and treatment with 100 mg/kg and 500 mg/kg of colocasia resulted in significant reduction to 41.82 g/l and 48.66 g/l respectively (Fig. 6).

3.7 Serum Albumin Levels

Administration of phenyl hydrazine significantly reduced the serum albumin level of the albino rat from 37.67 g/l to 24.46 g/l and treatment with 100 mg/kg and 500 mg/kg of *Colocasia esculenta* resulted in to 19.2 g/l and 21.9 g/l (Fig. 7).

4. DISCUSSION

Anemia is a disease associated with shortage of red blood cells, it can occur by several causes one of which is due to chemical substance like drug and other toxins example is phenyl hydrazine. Phenyl hydrazine aside its hemolytic effect on rat was reported by Merle et al. and Criswell et al. [6,7] to cause liver and kidney toxicity (because of their involvement in the clearance of hemolytic product like heme) and affect their biochemical parameters like creatinine, bilirubin, urea, albumin and total protein. On the other hand plant extracts like *Colocasia esculenta* have been discovered to restore normality to the effect of this toxic substance on said biochemical parameters.

The unconjugated bilirubin (a product of the heme portion of red blood cell that has undergone hemolysis) levels of the phenyl hydrazine treated rats experienced a significant increase compared to the normal group (untreated group). This is as a result of increased hemolysis (breakdown of red blood cell) to produce bilirubin whichis beyond the albumin (a plasma protein) binding capacity as reported by Cuperus et al. and Wang et al. [22,23]. This situation leads to increased level of unconjugated bilirubin level in the phenyl hydrazine treated rat. Increased unconjugated bilirubin level in the phenyl hydrazine treated rat also result in increased conjugated bilirubin level after binding to free serum albumin which takes the free unconjugated bilirubin to the liver for glucuronidation (also known as conjugation) [22].

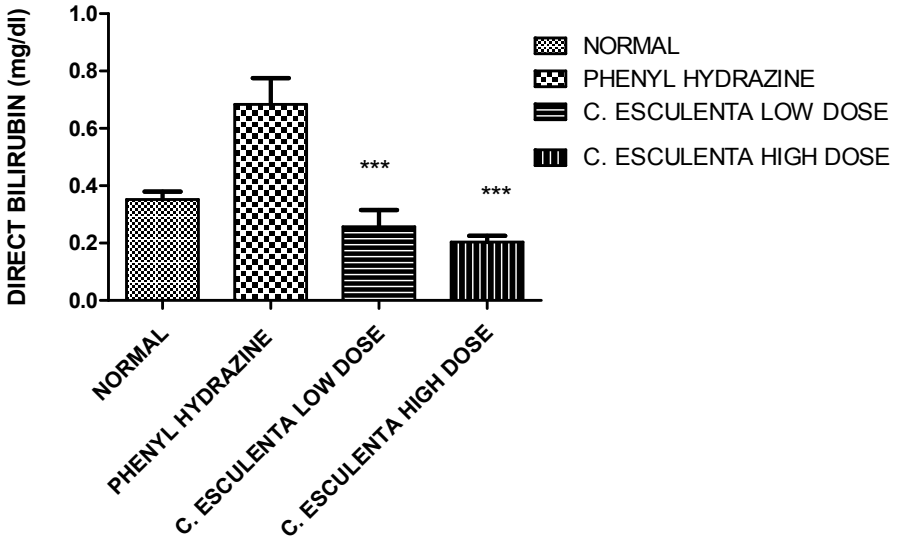

Fig. 1. Effects of *Colocasia esculenta* on direct bilirubin level in phenyl hydrazine induced anemic rat

** - significant value at p < 0.05 relative to the phenyl hydrazine treated group*

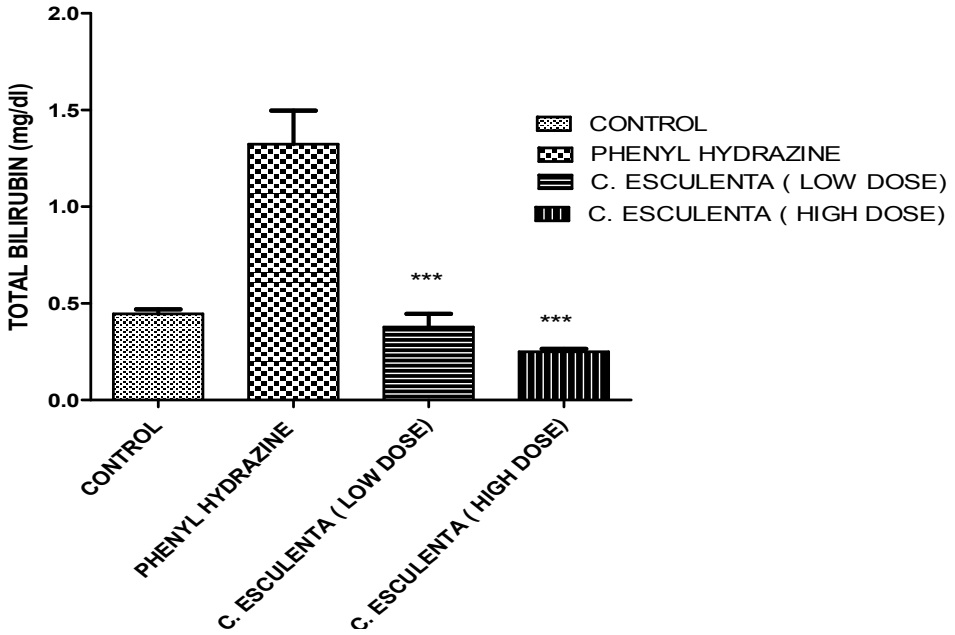

Fig. 2. Effect of *Colocasia esculenta* on total bilirubin level in phenyl hydrazine induced anemic rat

** - significant value at p < 0.05 relative to the phenyl hydrazine treated group*

The increased conjugated bilirubin level explains the reason for the reduced serum albumin level in the phenyl hydrazine treated rat because a part of the duty of the serum albumin is binding to free unconjugated bilirubin to enable easy conjugation in the hepatocyte and this was supported by Fukui et al. [24] in his work about the inverse relationship between conjugated bilirubin and serum albumin (meaning with increased amount of conjugated bilirubin there is

reduced albumin). Also the increased unconjugated bilirubin level in the phenyl hydrazine treated rat could also be as a result of liver disease resulting in impaired elimination of serum bilirubinby glucuronidation [6]. The ability of the plant extract to significantly reduce the bilirubin level of the phenyl hydrazine induced anemic rat makes it a very viable plant for treatment of hemolytic anemia and useful in the treatment of liver toxicity [25].

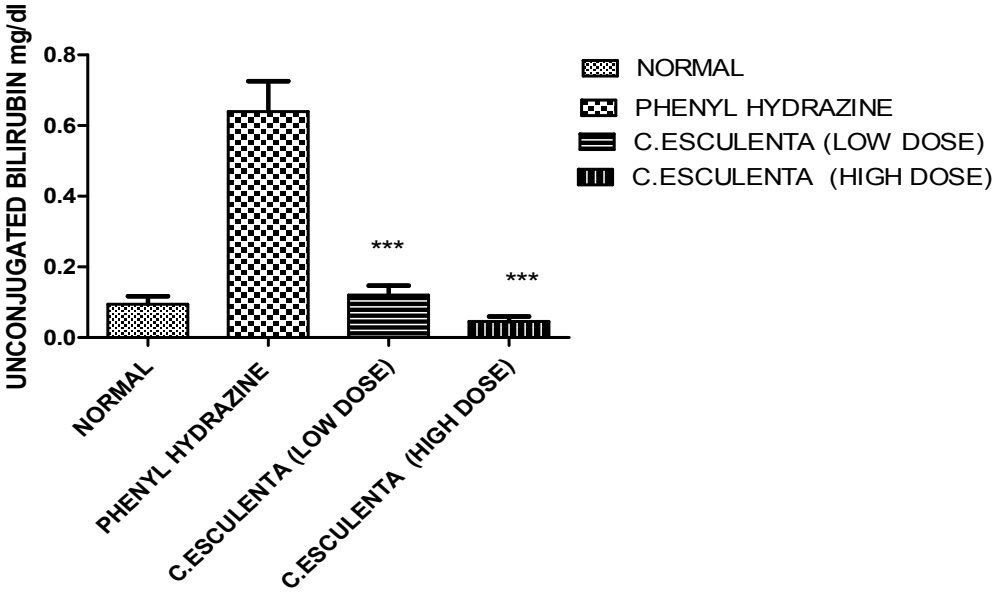

Fig. 3. Effect of *Colocasia esculenta* on unconjugated bilirubin level in phenyl hydrazine induced anemic rats

** - significant values at p < 0.05 relative to the phenyl hydrazine treated group*

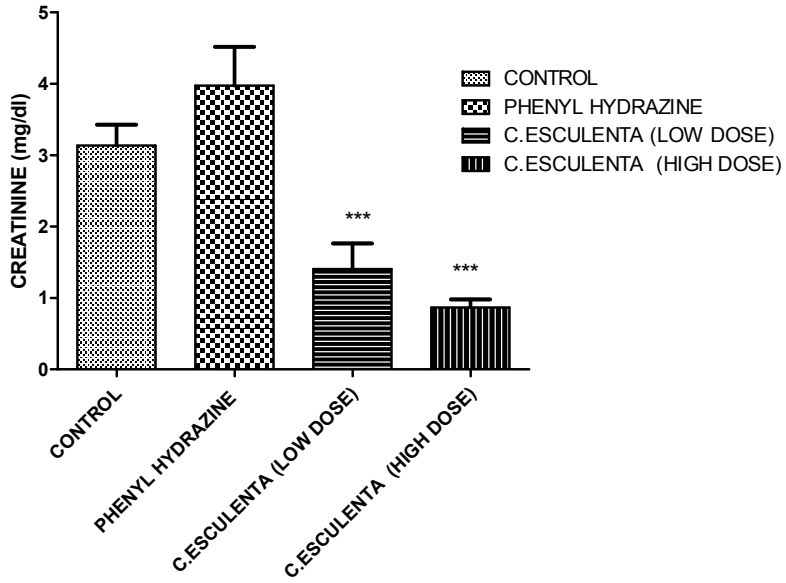

Fig. 4. Effect of *Colocasia esculenta* on creatinine level in phenyl hydrazine induced anemic rat

** - significant value at p < 0.05 relative to the phenyl hydrazine treated group*

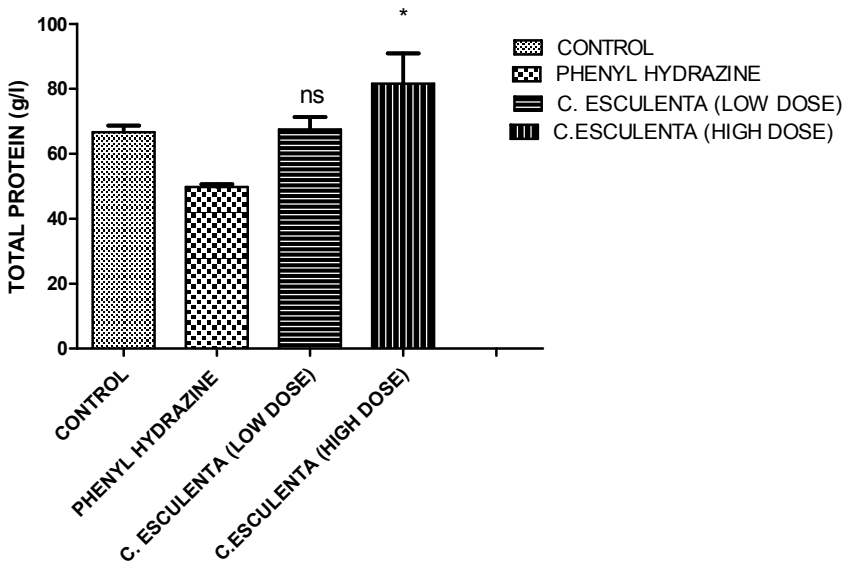

Fig. 5. Effect of *Colocasia esculenta* on total protein level in phenyl hydrazine induced anemic rat

**- significant values at p < 0.05 relative to the phenyl hydrazine treated group, ns – not significant at p < 0.05 relative to the phenyl hydrazine treated group*

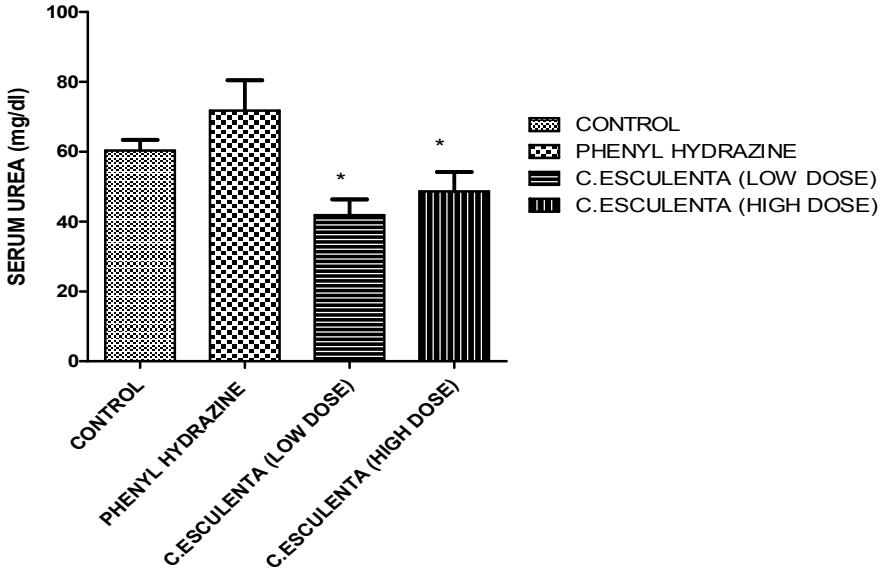

Fig. 6. Effect of *colocasia esculenta* on serum urea level in phenyl hydrazine induced anemic rats

**- significant values at p<0.05 relative to phenyl hydrazine treated group*

The significantly increased levels of creatinine in the phenyl hydrazine treated rats could be as a result of increased tissue breakdown caused by administration of phenyl hydrazine [26] and creatinine levels as a good assessment of kidney function (by how well it gets rid of waste product like creatinine) could show impaired ability of the kidney to get rid of it as a waste product due to damage or injury in phenyl hydrazine treated rat [6]. Considering the fact that the plant extract was able to reduce the creatinine level very significantly shows the plant at high and low

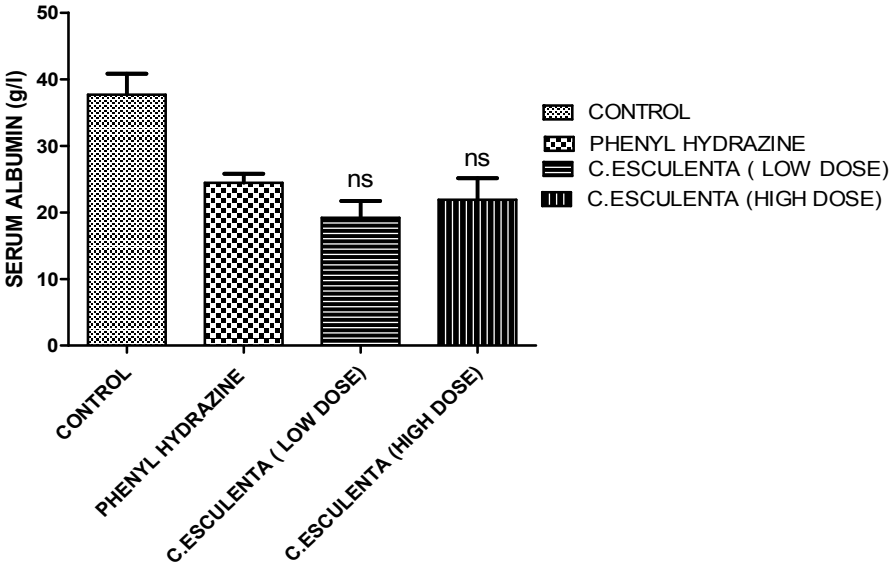

Fig. 7. Effect of *Colocasia esculenta* on serum albumin level in phenyl hydrazine induced anemic rat

ns- non significant value at p < 0.05 relative to the phenyl hydrazine treated group

dosage have the ability not only to reduce the rapid break down of tissues but to reduce the toxic effect of phenyl hydrazine on the kidney and improve tubular excretion [27].

The significantly increased serum urea level of the phenyl hydrazine treated rat could explain the significant reduction in total protein level of the same group of rats (considering the fact that urea production is linked to protein and amino acid breakdown). Impaired kidney function could be another explanation for the increased serum urea value because the kidney is involved in the its clearance from the blood and this could be as a result of injury damage or toxicity [6,28]. After treatment with the plant extract there was a significant reduction in the urea compared to the phenyl hydrazine treated rat which proves the plant as a very useful remedy for reducing the destructive effect of phenyl hydrazine on the kidney as reported by Lewu et al. [29].

Significant reduction experienced in the serum albumin level of the phenyl hydrazine treated rats was as a result of an impaired liver (organ of involved in albumin production) function because of increased erythropoiesis caused by severe blood shortage [26]. Hemolytic product are cleared from the blood by the liver but when hemolysis occurs at a very rapid rate it could lead to liver stress(due to increased work of clearing hemolytic product like heme) and therefore impaired function that occurred after treatment of the rats with phenyl hydrazine [30]. The use of the *Colocasia esculenta* extract at low and high dose did not restore normality (using untreated control rat value as reference) to the albumin level of the rats this could be as a result of binding to unconjugated bilirubin for uptake to the liver for conjugationas reported by Schreuder et al. [31].

The breakdown of serum albumin and extensive loss of membrane cytoskeletal on treatment with phenyl hydrazine reported by Chakrabrati et al. [32] could also result in the reduced value of serum albumin in the phenyl hydrazine treated rat. Despite the persistence in reduced albumin level after treatment of the rats with *Colocasia esculenta* the total protein experienced an increase in values on treatment with colocasia exract even when albumin is a part of serum total protein [33]. This could be as a result of the high globulin (about 80% of colocasia's protein content [34]) content of *Colocasia esculenta* which can as well increase the globulin (one of major portion of serum total protein) content of the blood leading to increased serum protein [35, 36].

5. CONCLUSIONS

Based on the findings of the study, it can be deduced that aqueous extract of *Colocasia*

esculenta at all doses exhibits remarkable hepatoprotective and anti-anemia effects. This extract also revealed amelioration in biochemical parameters.

ETHICAL APPROVAL

All authors hereby declare that "principles of laboratory animal care" (nih publication no. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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